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(71) Applicant *(for all designated States except US)*: **UNIVERSITA' DEGLI STUDI DI FIRENZE** [IT/IT]; Piazza San Marco, 4, I-50121 Firenze (IT).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **PAPINI, Anna, Maria** [IT/IT]; Via G. Alessi, 2, I-50127 Firenze (IT). **CHELLI, Maio** [IT/IT]; Località Fornacina, 6, I-50064 Incisa Val d'Arno (Prov. of Firenze) (IT). **ROVERO, Paolo** [IT/IT]; Via G. Alessi, 2, I-50127 Firenze (IT). **LOLLI, Francesco** [IT/IT]; Viale Guidoni, 143, I-50127 Firenze (IT).

(74) Agent: **GERVASI, Gemma**; Notarbartolo & Gervasi, Corso di Porta Vittoria, 9, I-20122 Milan (IT).

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(54) Title: GLYCOPEPTIDES, THEIR PREPARATION AND USE IN THE DIAGNOSIS OR THERAPEUTIC TREATMENT OF MULTIPLE SCLEROSIS

(57) Abstract: Glycopeptides capable of identifying multiple sclerosis antibodies, and therefore useful as diagnostic tools or for the treatment of said pathology are described.

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Glycopeptides, their preparation and use in the diagnosis or therapeutic treatment of multiple sclerosis

Field of invention

The present invention refers to glycopeptides formed of 11 – 24 aminoacids capable of identifying multiple sclerosis antibodies and therefore useful as diagnostic tools or for the treatment of said pathology.

State of the art

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system highly invalidating and therefore of high social impact. It causes the degradation of the central nervous system white matter (myelin) bringing about serious damage in the transmission of nerve signals.

The etiopathogenesis of the disease has not yet been clearly defined, but it is hypothesised that an autoimmune process directed against the principal myelin proteins is an important pathogenic mechanism of the disease. The myelin damage is most probably due to the synergic action of the T cell response and the antibody response against myelin proteins and glycoproteins. In particular the autoantibodies can play a key role in the activation of macrophages, in demyelinization, and in the blocking of nervous conduction.

In a previous study [A.M. Papini *et al.*, Proceedings of the 10th International Congress of Immunology, Monduzzi Editore, International Proceeding Division Bologna, Italy (1998), pp. 1239-1244] the possibility of identifying MS specific antibodies through a glycosylated peptide constituted of a sequence of 21 aminoacids of the myelin oligodendrocytic glycoprotein (MOG) (from positions 30 to 50), and indicated by the formula Asn^{31}(Glc)hMOG(30-50) has been demonstrated.

The interest in developing new glycosylated peptides capable of carrying out the function of identifying said antibodies with greater efficiency and therefore useful both for the diagnosis and for the treatment of multiple sclerosis is evident.

Summary of the invention

The present invention refers to glycopeptides of 11 – 24 aminoacids containing a tetrapeptide of formula (*I*):

X-Asn(G)-Y-Z (I)

in which:

X = aminoacid carrying an amino or carboxylic group on the side chain;

Y = Pro, Gly;

G is a sugar

Z = Ala, Val, Ile, His

Detailed description of the invention

It has now been surprisingly found, and is a subject of the present invention, that short glycopeptides, constituted of 11 – 24 aminoacids, containing the above-defined tetrapeptide play a very efficient role in the recognition of the antibodies typical of MS and are therefore useful in its diagnosis or its therapeutic treatment.

According to the present invention, glycopeptides of formula (II) are preferred:

A-B-X-Asn(G)-Y-Z-C-D (II)

in which:

Y and G are as defined above;

A = 2 – 5 aminoacids or absent

B and C = trifunctional aminoacids forming a lactam bridge between each other by means of the respective side chains, or absent;

D = 5 – 15 aminoacids;

X = Glu, Asp, Lys, Arg, Orn, Dap;

Z = Ala, Val, Ile, His.

For trifunctional aminoacids forming a lactam bridge between each other as defined above, is meant, for example, the pair Dap-Asp or Asp-Dap, Dab-Glu or Glu-Dab, Orn-Asp or Asp-Orn, and the pair formed by other aminoacids, for example non-natural aminoacids, having analogous characteristics.

For sugar is preferably meant: mono and disaccharides of type Glc, GlcNAc, β-D-Glc*p*-(1→4)-D-Glc (cellobiose), etc.

For aminoacids, when not otherwise defined, is meant natural or non-natural aminoacids.

Obviously residues A, if present, and D may contain an appropriate alkyl spacer to lengthen the chain, where for alkyl spacer, in the sense used herein, is meant ω-aminoacids with linear alkyl chains (H_2N-$(CH_2)_n$-CO_2H where n is 2 – 6.

The presence of the formula (I) tetrapeptide as defined above, can induce a folding in the peptide conformation that can, for this reason, assume a "hook like" form (when the tetrapeptide is present in the terminal portion of the peptide) or "a hairpin like" form (when the tetrapeptide is present in the central portion of the peptide). These conformations allow an optimal binding of the patients' autoantibodies; this fact is essential for the unexpected properties of the peptides according to the invention.

Particularly preferred, according to the invention are the peptides represented by the following sequences:

1. H-Thr-Pro-Arg-Val-Glu-Arg-Asn(Glc)-Gly-His-Ser-Val-Phe-Leu-Ala-Pro-Tyr-Gly-Trp-Met-Val-Lys-OH
2. H-Thr-Pro-Arg-Val-cyclic[Dap-Arg-Asn(Glc)-Gly-His-Asp]-Val-Phe-Leu-Ala-Pro-Tyr-Gly-Trp-Met-Val-Lys-OH
3. H-Thr-Pro-Arg-Val-cyclic[Asp-Arg-Asn(Glc)-Gly-His-Orn]-Val-Phe-Leu-Ala-Pro-Tyr-Gly-Trp-Met-Val-Lys-OH
4. H-Ala-Lys-Thr-Ala-Lys-Asn(Glc)-Gly-His-Val-Glu-Ala-Ser-Gly-OH
5. H-Glu-Asn(Glc)-Pro-Val-Val-His-Phe-Phe-Lys-Asn-Ile-Val-Thr-Pro-Arg-Thr-Pro-OH
6. H-Asp-Asn(Glc)-Pro-Val-Glu-Ala-Phe-Lys-Gly-Ile-Ser-OH
7. H-Thr-Pro-Arg-Val-Glu-Arg-Asn(Glc)-Gly-His-Ser-HN-$(CH_2)_6$-CO-Val-Phe-Leu-Ala-Pro-Tyr-Gly-Trp-Met-Val-Lys-OH
8. H-Asp-Asn(Glc)-Pro-Val-Val-His-Phe-Phe-Lys-Asn-Ile-Val-$(\beta Ala)_3$-OH

The peptides as defined above can also contain an –HN-$(CH_2)_n$-CO_2H group in the C-terminus (where n = 2-6) so as to allow the attachment to a resin as requested for their practical use in diagnostics or in therapeutic treatments.

The peptides according to the invention can be prepared according to known methods for solid or liquid phase synthesis.

The solid phase method is particularly useful, well known to experts in the field, the basis of which is that the C-terminal residue is covalently bound to an appropriate solid support, for example polystyrene (Wang's resin), polystyrene-polyoxyethylene (TentaGel resin or PEG-PS) or polyethylene glycol and polyacrylamide co-polymers (PEGA resin), and the successive aminoacids are

added sequentially, through acylation of the amino group of the residue bound to the resin, for example through the symmetric anhydride of the following aminoacid, appropriately protected, where necessary, on the side chain. Upon completion of the synthesis the crude peptide is obtained by treating the resin with an appropriate acid, for example hydrofluoric acid or trifluoroacetic acid, and separated by precipitation in ethyl ether and successive lyophilisation. The peptide is finally purified using chromatographic techniques, such as for example preparative HPLC. It is also possible to maintain the synthetic peptide bound to the solid support (for example polystyrene-polyoxyethylene TentaGel resin or PEG-PS), carrying out the selective deprotection of the side chains with an appropriate reagent.

Alternatively, still according to known techniques, the attachment of the peptide to the appropriate support is achieved so as to form the corresponding conjugates, useful in diagnostics or in therapy. The preferred supports for this purpose include resins, insoluble in water, and completely compatible with organic liquids, such as: silica gel, cellulose, polyacrylate, sepharose and analogues, as well as the same resins normally used by experts in the field for the preparation of synthetic peptides, as for example Wang's resin, polystyrene-polyoxyethylene (TentaGel resin or PEG-PS) or polyethylene glycol and polyacrylamide copolymers (completely compatible with water) such as PEGA resin and more stable analogue resins such as POEPS (polyoxyethylene-polystyrene), POEPOP (polyoxyethylene-polyoxypropylene), as well as macroporous resins described for their interest for the solid phase glycosylation of peptides, such as SPOCC (PEG substituted with oxethane) [Rademann, J; Grøtli, M; Meldal, M; and Bock, K. *J. Am. Chem. Soc.* 1999, 121, 5459-5466] or derivatives thereof like $EXPO_{3000}$ (copolymer with tetrakis-[4-(3-methyl-oxethane-3-ylmethyl)-phenyl]-silane) [Tornøe, C.W.; and Meldal M. In: Peptides 2000, J. Martinez and J.A. Fehrentz (Eds.) EDK, Paris, France 2001].

Examples disclosing the preparation of some peptides according to the invention, are provided in the following for illustrative, non limiting purposes of the invention.

Example 1. Synthesis of a linear peptide

Preparation of H-Thr-Pro-Arg-Val-Glu-Arg-Asn(Glc)-Gly-His-Ser-Val-Phe-Leu-Ala-Pro-Tyr-Gly-Trp-Met-Val-Lys-OH

The resin Fmoc-Lys(Boc)-NovaSyn (0.5 g, 0.11 mmol/g) was made to swell in DMF for 1 hour at room temperature and packed into the glass column (150 × 15 mm) of a continuous flow, semiautomated synthesiser. After deprotection of the amino group present on the resin with a solution of 20% piperidine in DMF, for the insertion of each aminoacid the column was loaded with a solution containing Fmoc-aminoacid (2.5 eq., 0.137 mmol) dissolved in DMF (1.3 ml), and as activating reagent HOBt (2.5 eq, 0.137 mmol), TBTU (2.5 eq, 0.137 mmol) and NMM (3.75 eq, 0.205 mmol).

The following aminoacids were used in order:

1) Fmoc-Val-OH
2) Fmoc-Met-OH
3) Fmoc-Trp(Boc)-OH
4) Fmoc-Gly-OH
5) Fmoc-Tyr(tBu)-OH
6) Fmoc-Pro-OH·
7) Fmoc-Ala-OH·
8) Fmoc-Leu-OH
9) Fmoc-Phe-OH
10) Fmoc-Val-OH
11) Fmoc-Ser(tBu)-OH
12) Fmoc-His(Trt)-OH
13) Fmoc-Gly-OH
14) Fmoc-Asn(Glc)-OH (N^{α}-Fmoc-N^{γ}-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-L-Asn-OPfp)
15) Fmoc-Arg(Pmc)-OH
16) Fmoc-Glu(OtBu)-OH·
17) Fmoc-Val-OH
18) Fmoc-Arg(Pmc)-OH
19) Fmoc-Pro-OH·

20) Fmoc-Thr(tBu)-OH

Upon completion of synthesis, the resin was deprotected with 20% piperidine in DMF, filtered, washed with DMF, DCM and ether, and finally dried under vacuum. The crude peptide was obtained by treatment of the resin with a mixture of TFA/phenol/anisole/ethanedithiol (94:2:2:2) (10 ml), maintained with agitation for 30 minutes at 0 °C and at room temperature for 1.5 hours. The resin was filtered and washed with TFA and the filtrate concentrated to half volume under vacuum. The peptide was precipitated by treatment with cold ether. The precipitate obtained was lyophilised recovering 112 mg of crude peptide.

Deacetylation was attained by adding dropwise, a solution of NaOMe 0.1 M in MeOH, to achieve a pH = 12, to a solution of the peptide in anhydrous MeOH (15 ml) maintained with agitation in a nitrogen atmosphere. The NaOMe solution was prepared by adding metallic sodium (270 mg in ligroin (pet. ether), 117 mmol) to distilled MeOH (11 ml) in a nitrogen atmosphere. After 1 hour dry ice was added to the mixture until neutralised. The solution was concentrated giving the crude peptide, which was purified by semipreparative HPLC (HPLC purity greater than 97%).

Characterisation: ESI-MS: found: [M+3H]3+ m/z = 869.9, [M+2H]2+ m/z = 1304.2; calculated: PM *monoisotopic* = 2605.38.

The other linear peptides were prepared following the same method, but using the necessary aminoacids in the appropriate sequences.

Example 2. Synthesis of a cyclic peptide.

Preparation of H-Thr-Pro-Arg-Val-cyclic[Dap-Arg-Asn(Glc)-Gly-His-Asp]-Val-Phe-Leu-Ala-Pro-Tyr-Gly-Trp-Met-Val-Lys-OH

TentaGel resin SPHB-Lys(Boc)-Fmoc (1.00 g, 0.22 mmol/g) was treated as described in example 1. For each coupling 4 eq of Fmoc-aminoacids (0.88 mmol) dissolved in DMF (1.3 ml) were used, and as activating reagents HOBt (4 eq, 0.88 mmol) and TBTU (4 eq, 0.88 mmol,) and NMM (6 eq, 1.68 mmol,) were used.

The following aminoacids were used in the following order:

1) Fmoc-Val-OH
2) Fmoc-Met-OH
3) Fmoc-Trp(Boc)-OH

4) Fmoc-Gly-OH
5) Fmoc-Tyr(tBu)-OH
6) Fmoc-Pro-OH
7) Fmoc-Ala-OH
8) Fmoc-Leu-OH
9) Fmoc-Phe-OH
10) Fmoc-Val-OH
11) Fmoc-Asp(OAll)-OH
12) Fmoc-His(Trt)-OH
13) Fmoc-Gly-OH
14) Fmoc-Asn(Glc)-OPfp
15) Fmoc-Arg(Pmc)-OH
16) Fmoc-Dap(Alloc)-OH
17) Fmoc-Val-OH
18) Fmoc-Arg(Pmc)-OH
19) Fmoc-Pro-OH
20) Fmoc-Thr(tBu)-OH

Upon completion of synthesis, the allylic protective groups of the Dap and Asp side chains were selectively removed by treatment with a solution of Pd(PPh3)4 (3 eq) in 7.5 ml of CHCl3/AcOH/ NMM 37:2:1 in Argon atmosphere. The reaction mixture was stirred using a mechanical arm for 3 hours at room temperature. The resin was then filtered and washed three times with a solution of 0.5% DIPEA in DMF, three times with a solution of 0.05% sodium diethyl carbamate in DMF, three times with DMF and three times with DCM.

For the subsequent cyclysation reaction, the resin was made to swell in a flask in DMF for 1 hour. NMM (4 eq.) was then added and after 10 minutes, PyBOP (4 eq.). The reaction mixture was agitated, using a mechanical arm, for three days at room temperature. The resin was filtered and washed several times with DMF, DCM and Et2O and finally dried under vacuum.

Afterwards, the resin was deprotected of Fmoc by 20% piperidine in DMF, followed by detachment of the peptide and its isolation exactly as described in example 1.

Example 3. Synthesis of a resin-bound peptide.

Preparation of H-Thr-Pro-Arg-Val-Glu-Arg-Asn(Glc)-Gly-His-Ser-Val-Phe-Leu-Ala-Pro-Tyr-Gly-Trp-Met-Val-Lys-(βAla)$_3$-PEG-PS

The base resin PEG-PS, in amino form (1 g, 0.09 eq) was made to swell in DMF for 1 hour at room temperature and packed into the glass column (150 × 15 mm) of a continuous flow, semiautomatic synthesiser. For the coupling of the first aminoacid the column was loaded with a solution containing Fmoc-βAla-OH (4 eq., 0.36 mmol) dissolved in DMF (1.5 ml), and as activating reagents HOBt (4 eq., 0.36 mmol), TBTU (4 eq., 0.36 mmol) and NMM (6 eq, 0.54 mmol). The reaction was carried out for 1 hour and later, after the appropriate washes, followed by the deprotection of the amino groups, by treatment with 20% piperidine in DMF. The coupling cycle for βAla was repeated a further twice, and then continued in the same way for the coupling of a residue of Fmoc-Lys(Boc)-OH. Later, the construction of the entire peptide sequence was continued, exactly as indicated in example1. Following the final treatment with piperidine, the resin-peptide was deprotected by treatment with a mixture of TFA/phenol/anisole/ethanedithiol (94:2:2:2) (10 ml), with agitation for 30 minutes at 0 °C and at room temperature for 1.5 hours, scrupulously washed and dried under reduced pressure.

Example 4. Conjugation of a peptide to resin.

A free peptide, linear or cyclic, prepared as described in examples 1 and 2, respectively, was conjugated to sepharose resin preactivated with CNBr, according to the usual reaction protocols advised by the manufacturers in order to obtain a resin-peptide conjugate. The product thus obtained is useful as for example for the preparation of plates for the diagnosis or treatment of patients affected by MS [see also the following].

The present invention refers also to a kit comprising the glicopeptides according to the invention and useful for diagnostic purposes.

According to a preferred embodiment of the invention a kit as above said comprises:

- a microplate
- a buffer solution for adhering the peptide to the plate;
- a modified peptide according to the invention (lyophilised);

- FCS-buffer (10% FCS, 9g/l NaCl, Tween 20 0,05%);
- concentrated wash solution (20x concentrated);
- positive control serum;
- negative control serum;
- an antibody reacting with the antibody of Multiple Sclerosis [conjugate-(AP conjugated with anti-Igm)];
- a substrate (p-nitrophenylphosphate, disoidum salt);
- a substrate buffer (1 M diethanolamine buffer, pH 9.8);
- a stop solution (1 M sodium hydrate).

If preferred the buffer solution for adhering the peptide to the plate and the modified peptide according to the invention can be incorporated directly in the microplate.

Diagnostic use

For diagnostic use the glycopeptides of the invention were diluted and absorbed onto binding plastic in the wells of microtitre plates (ELISA systems). Patient serum or plasma was then added in a series of different concentrations (dilution series). The autoantibody specific for our products bound to the peptide absorbed onto the plastic. According to the technique known by experts in the field, ELISA (Enzyme Linked Immuno-Sorbent Assay), the autoantibody molecules bound to the glycopeptide are then evidenced through the binding of appropriate secondary antibodies, added to the ELISA plates, which recognise the immunoglobulin constant fragment. These secondary antibodies, conjugated with appropriate enzymes, can be visualised through a colourimetric reaction: the absorbance developed is proportional to the amount of specifically bound autoantibody. Quantitatively, the result is expressed as the antibody titre, defined as the reciprocal of the dilution factor in which no further reaction is observed.

The antibody titre, as defined above, was measured in different patients affected by multiple sclerosis; the glycoproteins according to the invention have shown higher antibody titres compared to those measured with known glycopeptides.

Therapeutic use

The peptides according to the invention, in free form or bound to appropriate resins, can be used for the treatment of patients affected by multiple sclerosis as,

thanks to their high specificity of antibody recognition, they can be used to neutralise and/or selectively remove the autoantibodies.

CLAIMS

1. Glycopeptides constituted of 11 – 24 aminoacids containing a formula (I) tetrapeptide

X-Asn(G)-Y-Z (I)

in which:

X = aminoacid carrying an amino or carboxylic group on the side chain;

Y = Pro, Gly;

G = sugar

Z = Ala, Val, Ile, His

2. The glycopeptides according to claim 1 in which the aminoacids are the natural or non-natural aminoacids.

3. The glycopeptides according to claims 1 and 2, of formula (II):

A-B-X-Asn(G)-Y-Z-C-D (II)

in which:

Y and G are as defined above;

A is a group of 2 – 5 aminoacids or is absent;

B and C are trifunctional aminoacids capable of forming a lactam bridge between each other by means of the respective side chains or are both absent;

D represents a group of 5 – 15 aminoacids;

X is an aminoacid chosen from the group comprised of: Glu, Asp, Lys, Arg, Orn, Dap;

Z is an aminoacid chosen from the group comprised of: Ala, Val, Ile, His.

4. The glycopeptides according to claim 3, in which B and C represent the pairs: Dap-Asp or Asp-Dap, Dab-Glu or Glu-Dab, Orn-Asp or Asp-Orn and the pairs formed by other aminoacids, having analogous characteristics.

5. The glycopeptides according to claims 1 – 4, in which the sugar is chosen from the group consisting of mono- and disaccharides such as β-D-glucopyranosyl (Glc), 2-acetylglucosamina (GlcNAc), cellobiose and analogues.

6. The glycopeptides according to claim 5, in which the sugar is β-D-glucopyranosyl (Glc).

7. The glycopeptides according to claims 1 – 6, represented by the following formulae:

H-Thr-Pro-Arg-Val-Glu-Arg-Asn(Glc)-Gly-His-Ser-Val-Phe-Leu-Ala-Pro-Tyr-Gly-Trp-Met-Val-Lys-OH

H-Thr-Pro-Arg-Val-cyclic[Dap-Arg-Asn(Glc)-Gly-His-Asp]-Val-Phe-Leu-Ala-Pro-Tyr-Gly-Trp-Met-Val-Lys-OH

H-Thr-Pro-Arg-Val-cyclic[Asp-Arg-Asn(Glc)-Gly-His-Orn]-Val-Phe-Leu-Ala-Pro-Tyr-Gly-Trp-Met-Val-Lys-OH

H-Ala-Lys-Thr-Ala-Lys-Asn(Glc)-Gly-His-Val-Glu-Ala-Ser-Gly-OH

H-Glu-Asn(Glc)-Pro-Val-Val-His-Phe-Phe-Lys-Asn-Ile-Val-Thr-Pro-Arg-Thr-Pro-OH

H-Asp-Asn(Glc)-Pro-Val-Glu-Ala- Phe-Lys-Gly-Ile-Ser-OH

H-Thr-Pro-Arg-Val-Glu-Arg-Asn(Glc)-Gly-His-Ser-HN-$(CH_2)_6$-CO-Val-Phe-Leu-Ala-Pro-Tyr-Gly-Trp-Met-Val-Lys-OH

H-Asp-Asn(Glc)-Pro-Val-Val-His-Phe-Phe-Lys-Asn-Ile-Val-$(\beta Ala)_3$-OH

8. The glycopeptides according to claims 1 – 7, containing an alkyl spacer in non-terminal positions.

9. The glycopeptides according to claim 8, in which said alkyl spacer is a group of formula -HN-$(CH_2)_n$-CO_2- wherein n is comprised between 2 and 6.

10. The glycopeptides according to claims 1 – 9, containing a further C-terminal – HN-$(CH_2)_n$-CO_2H group wherein n is comprised between 2 and 6.

11. Process for the solid-phase preparation of glycopeptides according to claims 1 – 10, in which:

a) the C-terminal residue is covalently bound to an appropriate solid support;

b) the successive aminoacids are added sequentially, through acylation of the amino group of the residue bound to the resin;

c) the crude peptide is recovered by treatment of the resin with an appropriate acid;

d) the peptide is purified resorting to chromatographic techniques;

e) the peptide is eventually attached to an appropriate solid support.

12. The process according to claim 11, in which the solid support of stage (a) is chosen from the group consisting of silica gel, cellulose, polyacrylate, sepharose and analogues, polystyrene (Wang's resin), polystyrene-polyoxyethylene (TentaGel resin or PEG-PS), copolymers of polyethyleneglycol and polyacrylamide (PEGA resin), POEPS (polyoxyethylene-polystyrene), POEPOP (polyoxyethylene-

polyoxypropylene), SPOCC (PEG substituted with oxethane) or derivatives thereof.

13. The process according to claim 12, in which the solid support of stage (e), if present, is chosen from the group consisting of silica gel, cellulose, polyacrylate, and sepharose.

14. Conjugates constituted by a resin, insoluble in water and completely compatible with organic fluids, and a glycopeptide according to claims 1 - 10.

15. The conjugates according to claim 14, in which the resin is chosen from the group consisting sepharose, cellulose and silica gel.

16. Plates for diagnosis containing the glycopeptides according to claims 1 – 10.

17. Diagnostic methods in which the free glycopeptides according to claims 1 – 10 are used.

18. Diagnostic methods in which the conjugates according to claims 14 e 15 are used.

19. A kit for the diagnosis of Multiple Sclerosis comprising:

- a microplate;
- a buffer solution for adhering the peptide to the plate;
- a modified peptide according to claims 1 – 10 (lyophilised);
- FCS-buffer (10% FCS, 9g/l NaCl, Tween 20 0,05%);
- concentrated wash solution (20x concentrated);
- positive control serum;
- negative control serum;
- an antibody reacting with the antibody of Multiple Sclerosis [conjugate-(AP conjugated with anti-Igm)];
- a substrate (p-nitrophenylphosphate, disoidum salt);
- a substrate buffer (1 M diethanolamine buffer, pH 9.8);
- a stop solution (1 M sodium hydrate).

20. A kit according to claim 19 wherein said buffer solution for adhering the peptide to the plate and said modified peptide are incorporated directly in the microplate.

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(71) Applicant *(for all designated States except US)*: **UNIVERSITA' DEGLI STUDI DI FIRENZE** [IT/IT]; Piazza San Marco, 4, I-50121 Firenze (IT).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **PAPINI, Anna, Maria** [IT/IT]; Via G. Alessi, 2, I-50127 Firenze (IT). **CHELLI, Mario** [IT/IT]; Località Fornacina, 6, I-50064 Incisa Val d'Arno (Prov. of Firenze) (IT). **ROVERO, Paolo** [IT/IT]; Via G. Alessi, 2, I-50127 Firenze (IT). **LOLLI, Francesco** [IT/IT]; Viale Guidoni, 143, I-50127 Firenze (IT).

(74) Agent: **GERVASI, Gemma**; Notarbartolo & Gervasi, Corso di Porta Vittoria, 9, I-20122 Milan (IT).

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Declaration under Rule 4.17:
— *of inventorship (Rule 4.17(iv)) for US only*

Published:
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(54) Title: GLYCOPEPTIDES, THEIR PREPARATION AND USE IN THE DIAGNOSIS OR THERAPEUTIC TREATMENT OF MULTIPLE SCLEROSIS

(57) Abstract: Glycopeptides capable of identifying multiple sclerosis antibodies, and therefore useful as diagnostic tools or for the treatment of said pathology are described.

WO 03/000733 A3

INTERNATIONAL SEARCH REPORT

national Application No
PCT/EP 02/06767

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/705 C07K17/08 G01N33/68 C07K14/47

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MAZZUCCO S ET AL: "A Synthetic Glycopeptide of Human Myelin Oligodendrocyte Glycoprotein To Detect Antibody Responses in Multiple Sclerosis and Other Neurological Diseases" BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, OXFORD, GB, vol. 9, no. 2, 18 January 1999 (1999-01-18), pages 167-172, XP004152593 ISSN: 0960-894X the whole document ---	1-20
A	WO 97 35879 A (IMMULOGIC PHARMA CORP) 2 October 1997 (1997-10-02) the whole document ---	1-20

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 May 2003	03/06/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Groenendijk, M

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/06767

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 00 12126 A (UNIV CALIFORNIA) 9 March 2000 (2000-03-09) See especially claim 9; page 3, lines 1-9; page 7, lines 8-10; page 8, lines 13-18	1-20
A	WO 99 57241 A (ARIMILLI SUBHASHINI ;CORIXA CORP (US); DESHPANDE SHRIKANT (US)) 11 November 1999 (1999-11-11) see particularly Fig. 1A and 1B	

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